CHAPTER-3
MATERIAL AND METHODS

Present study was conducted August 2010 to July 2012. In the proposed work, Studies of various benthic macro-invertebrates and physico-chemical parameters of the Kunda River were planed on monthly basis at selected four study sites. The samples were taken between 7:00 AM to 12:00 Noon throughout the study duration from all study sites. The physico-chemical analysis of water and sampling of benthic macro-invertebrates was performed as per methods given in Needham & Needham (1969), Pennak (1989), Tonapi (1980), Welch (1998) and APHA (2005). Water samples were collected in plastic container for and used for Physico-chemical analysis. Net of mesh size 500µm is used for collecting the benthic macro-invertebrates. The organisms are preserved immediately in 80% ethanol or 4% formaldehyde. These samples are returned to the laboratory for processing. The collected benthic macro-invertebrates were sorted and identified to the best taxonomic level.

About Khargone District

Khargone District is one among 50 Districts of Madhya Pradesh State, India. Khargone District Administrative head quarter is Khargone. It is is Located 288 km. East towards State capital Bhopal. It is biggest district in State by population.
Geography of Khargone District

It is Located at Latitude-21.8, Longitude-75.5. Khargone District is sharing border with Barwani District to the west, Dhar District to the North, East Nimar District to the East. Khargone District occupies an area of approximately 8030 square kilometres. Its in the 198 meters to 181 meters elevation range. This District belongs to Hindi Belt India.

Climate of Khargone District

It is too hot in summer. Khargone District summer highest day temperature is in between 32°C to 44°C. Average temperatures of January is 23°C, February is 25°C, March is 30°C, April is 34°C, May is 36°C.

DemoGraphics of Khargone District

Hindi is the Local Language here. Also People Speaks Bhil, devanagari. Khargone District is divided into 9 Tehsils, 559 Panchayats, 2354 Villages. Segaon Tehsil is the Smallest Tehsil by population with 68967 populations. Khargone Tehsil is the Biggest Tehsil by population with 305240 populations.

POLLUTION OF KHARGONE DISTRICT:

Khargone District of Main climate change region. Water of river is absorbed and water pollution amount is increase. It district in ago 2 years 65 Thousands Bikes registered and Modern time 85 Thousands to more bike registered and 2 years ago 826 cars was registered and in 2 years ago 200 cars registered it reason its district Noise pollution, Soil pollution and Water pollution and many more pollution also increases.
Khargone District climate is changed. Water of river is absorbed and water pollution amount is increased day by day. In the Kunda river reach 4 gutters and polluted water, every month 1 ton or its more waste material reach in Kunda River. Khargone district and its attached Panchayat also increased quantity of chlorides in water and yet increased disease of Allergy, And through chemical manure as sulphur reach in water sources. Many years ago pH of soil was 7 but current more quantity uses of chemical manure soil and water both polluted.

**STUDY AREA:**

**Description of Kunda River**

The Kunda River is a Main river of Khargone district. It is a tributary river of Narmada river. It is originated from forest, Amba and Sirvel village. River Kunda has a length of approximately 169Kms. and its catchment area of 3825sq.km. This river is situated in the west directions of M.P. and it flows from South to North through four block of Khargone district Bhagwanpura, Goganwa, Khargone and Kasrawad. On the Kunda River there are two Dam constructed Dejla-Devada dam & Vanihar dam. It provides drinking water for the Khargone city. There is on a Shiv temple and Ahilyaghat before Siddhi vinayak ganesh temple at the bank of Kunda River in Khargone. There are 7 stop dams is being constructed in last two years. These Stop dams provide drinking water & irrigation facility to Khargone District. Its water works water capacity is 20 crore litre. Its water holding capacity of 7 stop dams is 0.646 million cubic meters. Its capacity in stop water 1.5 million cubic meter and these stopdams are made in front of Kalika mata temple.

**Latitude 21°49’16” (DMS) N and Longitude (DMS) 75°36’4”E.**
Map -1. Location map showing the Kunda River and Four sampling points, Khargone, M.P. India.
1.1 Description of Sampling Station

Before finally fixing the sampling stations a general survey of River was made, samples were collected and estimated from various regions in which Kunda river flow. Accordingly and study areas were fixed.

Study Site-

The water samples would be collected from the various selected sampling station in the Kunda River which are as under.

1. STATION I:- DEJLA-DEVADA DAM

Dejla-Devada Dam envisages construction of a storage Reservoir near village Dejla Dewada, District Khargone, across river Kunda River, a tributary of Narmada basin. The Cathment capture area (CCA) of the dam is 9000 hectare and annual irrigation is 12500 hectare. The cost of the dam is Rs. 51.66 crore. The dam was approved by Planning Commission in the year 1983 for an estimated cost of Rs.1643 lakh (1983 price level).

The Dejla devada dam components are as under-

- An earthen dam 1560 m. long and of max. Height of 35.2 m. across river Kunda.
- A saddle earthen dam 210 m. long and of max. Height of 11.72 m. in the right flank.
• Masonry spillway (ogee) 400 m. long to pass the design flood of 2491.88 cumecs.
• Masonry head sluice on the left flank of dejla-devada dam.
• Lined main canal 32.5 km. long taking of from the left flank of the dam at RD 60 m. to irrigate CCA of 9000 hectare.

Its latitude 21°36'45" (DMS) N & longitude 75°37'30"E (DMS) E

2. STATION II: - CONFLUENCE WITH UNDRI RIVER

Undri River is a tributary river of Kunda river. This place is situated 12km away From the Dejla-Devada dam. At this village Undri river confluence in Kunda River this village is called Bagdhari. At this place Garhi-Galtar project has made, and its dam water holding capacity 7.50m. This provided Irrigation facility to near about 1157 km. hectare Land.

Its latitude 20°41’30” (DMS) N & longitude 75°52’15” (DMS) E

3. STATION III: - BARRAGE OF KHARGONE

Khargone District region was formerly known as West Nimar. Khargone is situated on the bank of Kunda River. This place has construct Barrage on the River Kunda. This Barrage has Total forty gates and it gate attach Plates. Its Length is 165 feet and its water storage capacity is two million cubic meters. Khargone District lies in southwestern boundary of Madhya Pradesh. It has acquired an area of 8030 sq km. It is surrounded by
Dhar, Indore & Dewas in the North, Maharashtra in the South, Khandwa, Burhanpur in the East and Barwani in the West. District forms almost central section of Narmada valley which is bordered by Vindhyachal ranges in North & Satpura ranges in South. Narmada is the main river flowing about 100 km in the District and Kunda River is a tributary river of Narmada river. Its **Elevation / Altitude:** 198 - 181 meters. above Sea level.

**Its latitude 20°22’ and 22°35’ N & longitude 74°25’ and 76°14’ E**

4. **STATION IV: - SIPTAN (CONFLUENCE OF KUNDA AND VEDA RIVER)**

Siptan is a Village in Kasrawad Tehsil in Khargone District of Madhya Pradesh State, India. It belongs to Indore Division. It is located 22 km. towards North from District head quarters Khargone. Siptan is terminal point of Kunda river, At this point Kunda river confluence with the Veda River. After this place Kunda river is called Veda River. Siptan is near the village named Bhulgori. Siptan catchments area is about 798.10sq.m. Its **Elevation / Altitude:** 198 meters. above Sea level.

**Its latitude 21°41’30” (DMS) N & longitude 75°41’30” (DMS) E**
STATION- I DEJLA- DEVADA DAM

STATION- II CONFLUENCE WITH UNDRI RIVER
STATION-III BARRAGE OF KHARGONE

STATION- IV SIPTAN (CONFLUENCE OF KUNDA AND VEDA)
1.2 PHYSICO-CHEMICAL ANALYSIS OF WATER:

Water analysis

The water samples were collected from the four selected sampling stations viz., Station I, Station II, Station III and Station IV in the Kunda River for the period of two year from August 2010 to July 2012. In the analysis of the physico-chemical properties of water, standard method prescribed in limnological literature were used. Temperature, pH, Transparency and Dissolved Oxygen were determined at the site while other parameters like Biochemical oxygen demand, Total Hardness, Alkanity, Chloride, Nitrate and Phosphate were determined in the laboratory. The Physico-Chemical parameters were determined by standard methods of Golterman (1978), Welch (1998), APHA (2005).

PHYSICAL ANALYSIS OF WATER:

1. TEMPERATURE

Temperature readings are used in the calculation of various forms of alkalinity in studies of saturation and stability with respect to calcium carbonate.

In limnological studies, water temperature, as a function of depth is often required. Elevated temperatures resulting from discharge of heated water may have significant ecological impact. Identification of source of water supply such as deep wells often is possible by temperature measurements alone.
Method

Normally, temperature may be measured with any good mercury filled Celsius-thermometer. As a minimum, a thermometer should have a scale marked for every 0.1°C, with marking etched on the capillary glass and minimal thermal capacity to permit rapid equilibrium.

The temperature of deep water required for limnological studies was measured with a reversing thermometer. The temperature of surface and sub-surface water was recorded by drawing water samples with the help of a sample or by dipping the thermoprobe to the desired depth. Since only the physico-chemical characterization of the water bodies was intended, not the detailed study of thermal regime, simple mercury thermometer was used to note the water temperature usually at the time of sampling that was morning.

2. Hydrogen ion concentration (pH)

Principle:

The pH of the solution refers to its Hydrogen ion activity and is expressed as logarithm of reciprocal of hydrogen ion concentration in mole per litre at given temperature. pH is the “intensity” factor of acidity, pH scale ranges from 0-14 with mid point 7 as a neutral point below and above is acidic or alkaline respectively.

Nearest equation governing the effect of concentration of ions and formation of single electrode potential across the glass membrane is the basic concept of pH measurement. pH is an important factor in water
chemistry since it enters into the calculation of acidity and alkalinity and process such as coagulation, disinfection, softening and corrosion control.

**pH can be measured**

1. Colorimetrically;
2. Electrometrically,
3. pH paper.

**Colorimetric method**

**Procedure**

10 ml of sample is taken in a test tube and 4-5 drops of universal pH indicator was added to it. It was then mixed well by shaking. Now the colour developed is compared with colour glass standards and colour nearest to standards was selected and the pH value is noted.

**3. Transparency**

Turbidity in terms of transparency was determined by Secchi disc method. A circular metal disc of 20 cm in diameter was prepared with two white and two black equal quadrants alternatively on, the upper surface. To eliminate the possibility of reflection of light from the other side it was painted black on the middle of the upper surface. A hook was soldered to tie a long wide plastic string and an opposite surface a heavy iron rod was fixed. This extra weight helped in the immersion of disc in water. The disc was dipped into water with the help of tagged thread and the point of its disappearance was noted. It was then gradually lifted till also disappeared.
The point of its reappearance was recorded. The turbidity was calculated by these two readings.

\[
\text{Transparency (cm)} = \frac{d_1 + d_2}{2}
\]

Where, 
\(d_1\) = depth when secchi disc disappeared
\(d_2\) = depth when secchi disc reappeared.

CHEMICAL ANALYSIS OF WATER

4. Dissolved Oxygen (DO)

It is of paramount importance to all living organisms and is considered to be the lone factor, which to a greater extent can reveal the nature of whole aquatic system.

**Principle:**

The Mangnous Sulphate reacts with the alkaline Potassium Hydroxide, which in the presence of oxygen gets oxidized to brown colour compound. In the strong acid medium mangnaic ions are reduced by iodine ions, which gets converted to iodine equivalent to the original concentration of oxygen in the sample. The liberated iodine can be titrated against sodium thiosulphate using starch as an indicator.

\[
\text{MnSO}_4 + 2 \text{KOH} \rightarrow \text{Mn(OH)}_2 + \text{K}_2\text{SO}_4
\]

\[
2 \text{Mn(OH)}_2 + \text{O}_2 \rightarrow 2 \text{Mn(OH)}_3
\]
$$\text{Mn(SO}_4\text{)}_2 + 2 \text{KI} \rightarrow \text{MnSO}_4 + \text{K}_2\text{SO}_4$$

$$2 \text{NaS}_2\text{O}_3 + \text{I}_2 \rightarrow \text{Na}_2\text{S}_4\text{O}_6 + 2 \text{NaI}$$

**Procedure**

The sample is collected in 300 ml BOD bottle. 2 ml mangnous sulphate (36%) and 2 ml alkaline potassium iodide solution (100 g KOH and 50g KI in 200 ml distilled water) was added to the sample and was shaked. The precipitate was allowed to settle, then 2 ml conc. H$_2$SO$_4$ is added, was shaked well till the precipitate dissolved. Titrated the liberated I$_2$ with 0.025 Na$_2$S$_2$O$_3$ (Sodium thiosulphate) using starch as an indicator.

**Calculation**

$$\text{Dissolved Oxygen in mg/l} = \frac{V_1 \times N \times 8 \times 1000}{V_2}$$

Where

$$V_1 = \text{Volume of Na}_2\text{S}_2\text{O}_3$$

$$N = \text{Normality of Na}_2\text{S}_2\text{O}_3$$

$$V_2 = \text{Volume of sample used}$$

**5. Biological Oxygen Demand (BOD)**

Biological Oxygen Demand was measured of the degraded organic material present in water sample, and defined as the amount of oxygen required by the micro-organism in stabilizing the biologically degradable organic matter under aerobic conditions.
**Principle**

The principle of the method involves measuring the difference of the oxygen concentration between the sample before and after incubation for 3 days at 27°C.

**Procedure**

Two BOD bottles were taken and filled fully with sample up to the neck. One of the bottles was placed in incubator for 3 days at 27°C. And in the second BOD bottle, initial BOD was determined by fixing it with 1 ml of Alkali azide and 1 ml of Magnous Sulphate. Then 2 ml of conc. H₂SO₄ was added so that the precipitate gets settle down. Now 200 ml of this sample was taken and tritrated with sodium thiosulphate by adding starch as an indicator, till the sample becomes colourless. BOD bottle is taken out after 3 days from the incubator and the final BOD is determined using the same procedure.

**Calculations**

\[ \text{BOD in mg/l} = (D₀ - D₃) \]

Where

\[ D₀ = \text{Initial } D₀ \text{ in the sample} \]

\[ D₃ = \text{Final } D₀ \text{ after 3 days at } 27^°C. \]

6. **Total Hardness**

Total hardness in water is the sum of the concentration of alkaline earth metal cations (Ca⁺⁺, Mg⁺⁺) etc.
Principle

Erichrome black ‘T’ forms wine red complex compound with metal ion. The di-sodium salt EDTA (ethylene diamine tetra acetic acid) extracts the metal ions from the dye metal ion complex as colourless chelate complexes leaving a blue coloured aqueous solution of the dye.

Method

50 ml of sample was taken and to it 2 ml Ammonia buffer solution and a pinch of Erichrome Black “T” is added as an indicator. Titrated it with EDTA solution until blue colour appears.

Calculation

Total Hardness in mg/l = \( \frac{\text{ml of titrate} \times 1000}{\text{Volume of sample}} \)

7. Alkalinity

Principle:

The estimation if based on simple acidimetric titration using different indicators which work in alkaline pH range (above 8.2) or in acidic range below (6.0).

The alkalinity of water is due to presence of carbonate, bicarbonate and hydroxide compounds of calcium, magnesium, sodium and potassium etc.

Phenolphthalein and methyl orange are the indicators commonly used for alkalinity titrations. To determine the carbonate alkalinity or hydroxide alkalinity 100 ml of water after adding 2-3 drops of phenolphthalein
indicator was titrated against N/50 till the pink colour was disappeared. The amount of acid used gave the value of carbonate or hydroxide.

For bicarbonate determination, methyl orange indicator (2-3 drop) was added to the same beaker and the titrate (N/50 H₂SO₄) was mixed from the same pipette till the end point reached. Showing bicarbonate present in the sample.

**Calculation:**

\[
\text{Mg CaCO}_3 \text{ per liter} = \frac{\text{Total standard acid} \times 100}{\text{Ml of sample}}
\]

8. **Chloride**

Chloride is usually present in low concentration in natural waters and play metabolically active role in photolysis of water. Their high concentrations are considered as the indicators on pollution from animal origin as animal excretion contains lots of chloride salts. Free chloride, which is commonly used as a disinfectant for drinking and wastewater, soon gets either converted into chlorides or combines with matter to form toxic compounds.

**Principle**

In portable water the salty taste was produced by chloride ion concentration. The chloride ions are determined by the titration with standard silver nitrate solution in which silver chloride precipitates out. The end point of the titration is indicated by the formation of red silver chromate
from excess silver nitrate. The potassium chromate is used as an indicator in neutral to slightly alkaline solution.

**Method**

50 ml of sample was taken and 1 to 2 drops of potassium chromate solution was added as an indicator and titrated with silver nitrate solution until colour appears.

**Calculations**

\[
\text{Chloride in mg/l} = \frac{\text{Reading of Titrate} \times 500}{\text{Volume of sample}}
\]

**9. Nitrate**

Nitrate is the most oxidized form of nitrogen and is an important plant nutrient. In a system approaching higher trophic levels the organic material or metabolic waste descend to deeper waters where, nitrogen which does not get lost to the sediments is remineralised to nitrates via bacterial oxidative process by nitrifying bacteria.

**Principle**

The reaction between nitrate and phenol disulphonic acid results in formation of 6 nitro 1, 2, 4 Phenol disulphonic acid which on conversion to the alkaline salt yield yellow colour.

**Method**

100 ml of sample was taken. It was heated to dryness in water bath, 2 ml of phenol di-sulphonic acid 100 ml of distilled water was added. Now 6-7
ml of Ammonium Solution was again added. Yellow color appears which can be measured spectrophotometrically at 410 nm and was compared against the calibration curve drawn for various known concentrations.

10. Phosphates

Phosphates, which are readily taken up by the phytoplanktons, often, deplete rapidly becoming the first limiting nutrient. In eutrophic lakes high phosphorus content supports an increased level of primary production.

Principle

Phosphate in an acidified ammonium molybdate solution produces blue colour with stannous chloride is added. This colour is measured by spectrophotometer at 690nm.

Method

50 ml of sample was taken and 2 ml of ammonium molybdate solution and 1 ml of stannous chloride solution was added to it. The blue colour appears for some time and then the reading is taken on spectrophotometer at 690 nm and compared against the calibration curve drawn for various known concentration.

Calculation

\[
\text{Graph reading} \times 1000 \times \text{dil}^n \times \text{factor} \times \frac{1}{\text{Volume of sample}}
\]
BIOLOGICAL ANALYSIS

Collection and identification of Benthic macro-invertebrates with the help of standard books Needham & Needham (1969), Pennak (1989), Tonapi (1980), Welch (1998) and A.P.H.A. (2005). Benthic communities along the river were sampled monthly from August 2010 to July 2012 at each of the four stations using D-net and Ekman grab (for deeper sites). The samples were collected by a bottom kick net (500 µm mesh). The samples were taken from an area of nearly 100 m2 in order to include all possible microhabitats at each station. In some areas with the presence of large stones, these were first picked out and washed into the kick net to remove pupae and other attached macro-invertebrates. In addition, macro-invertebrate samples were separated from the macrophytes and the sediment using sieves (250 µm). All the animals collected were immediately fixed in formaldehyde (4%) in the field and then transferred to 70% ethyl alcohol. The macro-invertebrates were sorted, identified to the lowest possible taxon (species, genus or families) and counted under a stereomicroscope.

The benthic macro invertebrate samples were preserved in 80% ethanol before laboratory identification. In the laboratory, the sample was then rinsed with tap water to remove the preservative and then sorted out into major taxa. The sorted organisms were stored in 10 ml glass bottle containing 70% ethanol for preservation and for subsequent identification.

Benthic macro-invertebrates organisms and residual debris retained in the screen were placed on ice. Within 24 hours after collection, the organisms were handpicked, sorted into major taxonomic groups and preserved in 75% ethanol. The organisms were identified from standard
taxonomic keys (e.g. Pennak 1989). A complete list of the taxa taken at each station is available from the senior author.

Data analysis:

Diversity was computed using the Shannon-Wiener Index or H’ (Mason 1996). Number of taxa (taxa richness or Taxa S) was measured by counting the number of macro-invertebrates families found in the samples (USEPA 1997).

Shannon-Wiener species diversity index:

A diversity index is a mathematical measure of species diversity in a community. Diversity indices provide more information about community composition than simply species richness (i.e., the number of species present); they also take the relative abundances of different species.

Importance:

Diversity indices provide important information about rarity and commonness of species in a community. The ability to quantify in this way is an important tool for biologists trying to understand community structure.

Methods:

The Shannon-Wiener diversity index (H) is another index that is commonly used to characterize species diversity in a community. Shannon’s index accounts for both abundance and evenness of the species present. The proportion of species I relative to the total number of species (pi) is calculated, and then multiplied by the natural logarithm of this proportion (lnpi). The resulting product is summed across species, and multiplied by -1:
It has been calculated as

\[ H' = -\sum_{i=1}^{R} p_i \ln p_i \]

Where \( H' \) = Shannon and Weaver Index.

\( P_i = \frac{n_i}{N} \) (\( n_i \) = number of individuals of the species)

\( p_i \) is the proportion of characters belonging to the \( i \)th type of letter in the string of interest. In ecology, \( p_i \) is often the proportion of individuals belonging to the \( i \)th species in the dataset of interest. Then the Shannon entropy quantifies the uncertainty in predicting the species identity of an individual that is taken at random from the dataset.

**National Sanitation Foundation Water Quality Index (NSFWQI):**

National Sanitation Foundation Water Quality Index (NSFWQI) is an excellent management and general administrative tool in communicating Water quality information. This index has been widely field tested and applied to data from a number of different geographical areas all over the world in order to calculate Water Quality Index of various water bodies critical pollution parameters were considered.

The Mathematical expression for NSFWQI is given by:

\[ \text{NSFWQI} = \sum_{i=1}^{p} W_i I_i \]

Where \( I_i = \) is the Sub-index for \( i \)th Water quality Parameters
Wi = is the Weight (in terms of importance) associated with ith Water quality Parameter.

p = is the number of Water quality parameters.

**CORRELATION COEFFICIENT: -**

**Statistical analyses**

The Pearson Correlation Coefficients was used to determine the level of significance of the relationship among benthic macro-invertebrate metrics and physico-chemical parameters

- The correlation coefficient computed from the sample data measures the strength and direction of a relationship between two variables.
- The range of the correlation coefficient is.
- -1 to +1 and is identified by $r$.

**Formula for correlation coefficient**

The Karl pearson’s correlation is calculated by formula

$$r_{xy} = \frac{N \sum XY - \sum X \sum Y}{\sqrt{[N \sum X^2 - (\sum X)^2][N \sum Y^2 - (\sum Y)^2]}}$$

Where

- $r = \text{coefficient of correlation}$
- $N = \text{no of months.}$
- $X \text{ & } Y = \text{variables.}$

$r$ may be positively or negatively correlated with each other. If the value of $r$ is greater than 0.6, it indicate that the variables are dependent with each other.